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# Utilization of C-C Chemokine Receptor 5 by the Envelope Glycoproteins of a Pathogenic Simian Immunodeficiency Virus, $SIV_{mac}239$

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**We examined chemokine receptors for the ability to facilitate the infection of CD4-expressing cells by viruses containing the envelope glycoproteins of a pathogenic simian immunodeficiency virus, $SIV_{mac}239$. Expression of either human or simian C-C chemokine receptor CCR5 allowed the $SIV_{mac}239$ envelope glycoproteins to mediate virus entry and cell-to-cell fusion. Thus, distantly related immunodeficiency viruses such as SIV and the primary human immunodeficiency virus type 1 isolates can utilize CCR5 as an entry cofactor.**

Human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) induce AIDS in humans, and simian immunodeficiency virus (SIV) can induce AIDS-like illness in Old World monkeys (4, 14, 18, 25, 40). Isolates of HIV-1, the major cause of AIDS in humans, have been phylogenetically segregated into groups M and O (50). Within the larger group, M, are several diverse clades of HIV-1. HIV-2 and SIV form a distinct group of phylogenetically and antigenically related viruses (14, 18, 32, 62).

AIDS induced by HIV-1 or HIV-2 in humans or by SIV in monkeys is characterized by the depletion of $CD4^+$ T lymphocytes, which represent a major target of viral infection in vivo (22). Infection of other $CD4^+$ cell types, such as monocytes in the blood, macrophages in the tissues, and microglial cells in the brain, has been suggested to be important for the pathogenesis of primate immunodeficiency viruses in the central nervous system and in the lungs (19, 26, 27, 37, 55). Certain populations of dendritic cells in the blood and tissues may also be infected by these viruses (53, 63).

The tropism of primate immunodeficiency viruses for $CD4^+$ cells is explained by the utilization of the CD4 glycoprotein as a primary receptor for virus entry into the cell (16, 36, 45). The viral envelope glycoproteins, which mediate virus entry, consist of the gp120 exterior envelope glycoprotein and the gp41 transmembrane glycoprotein (2, 56). The gp120 glycoprotein binds the CD4 molecule, following which the concerted action of the gp120 and gp41 glycoproteins results in the fusion of viral and cellular membranes (28, 38, 46, 61). The interaction of the viral envelope glycoproteins expressed on the infected cell surface with adjacent $CD4^+$ cells results in the formation of syncytia by an analogous process (42, 60).

Host cell factors in addition to CD4 have been suggested to determine the efficiency of primate immunodeficiency virus envelope glycoprotein-mediated membrane fusion. Some human and animal cells were shown to be resistant to HIV-1, HIV-2, or SIV infection and syncytium formation even when human CD4 was expressed on the cell surface (2a, 45, 47). HIV-1 variants have been identified that infect either primary monocytes/macrophages or immortalized $CD4^+$ cell lines in addition to primary T lymphocytes. The macrophage-tropic primary HIV-1 viruses cannot infect T-cell lines, laboratory-adapted viruses cannot infect primary monocytes/macrophages, and T-cell line-tropic primary viruses exhibit dual tropism for these cell types (8a, 24a, 57b). Changes in the viral envelope glycoproteins, in particular in the third variable (V3) region of the gp120 exterior envelope glycoprotein, determine these phenotypes (7–11, 30, 51, 59, 64, 65). Recently, it has been shown that HIV-1 entry and fusion require the expression of specific members of the chemokine receptor family on the target cell membrane in addition to CD4. Most T-cell line-tropic primary viruses and laboratory-adapted viruses utilize a CXC chemokine receptor called CXCR4 (also called LESTR, HUMSTSR, or fusin) (20, 23, 24, 44), while most macrophage-tropic primary HIV-1 viruses use the C-C chemokine receptor CCR5 (1, 12, 17, 21). The natural ligands for these chemokine receptors (SDF-1 for CXCR4 and RANTES, MIP-1α, and MIP-1β for CCR5 [54, 57]) inhibit the infection of the particular HIV-1 variants that utilize these molecules for entry (5, 15, 50a). The structure of the V3 loop on the HIV-1 gp120 envelope glycoprotein is a major determinant of which chemokine receptor can be used as an entry cofactor (12).

The primate immunodeficiency viruses share common features of envelope glycoprotein organization, with similar locations of the variable and conserved regions, cysteine residues, and residues important for CD4 binding (39, 49a, 50). Significant differences, however, exist between the HIV-1 and SIV envelope glycoproteins. None of the epitopes on the HIV-1 gp120 glycoprotein, the major target for neutralizing antibodies, are retained on the gp120 glycoprotein of HIV-2 or SIV (31, 34). The naturally arising $SIV_{mac}$ determinants of primary monocyte/macrophage tropism reside within the *env* gene but do not involve envelope glycoprotein regions equivalent to the HIV-1 V3 loop (3, 48, 49). Furthermore, these envelope gly-

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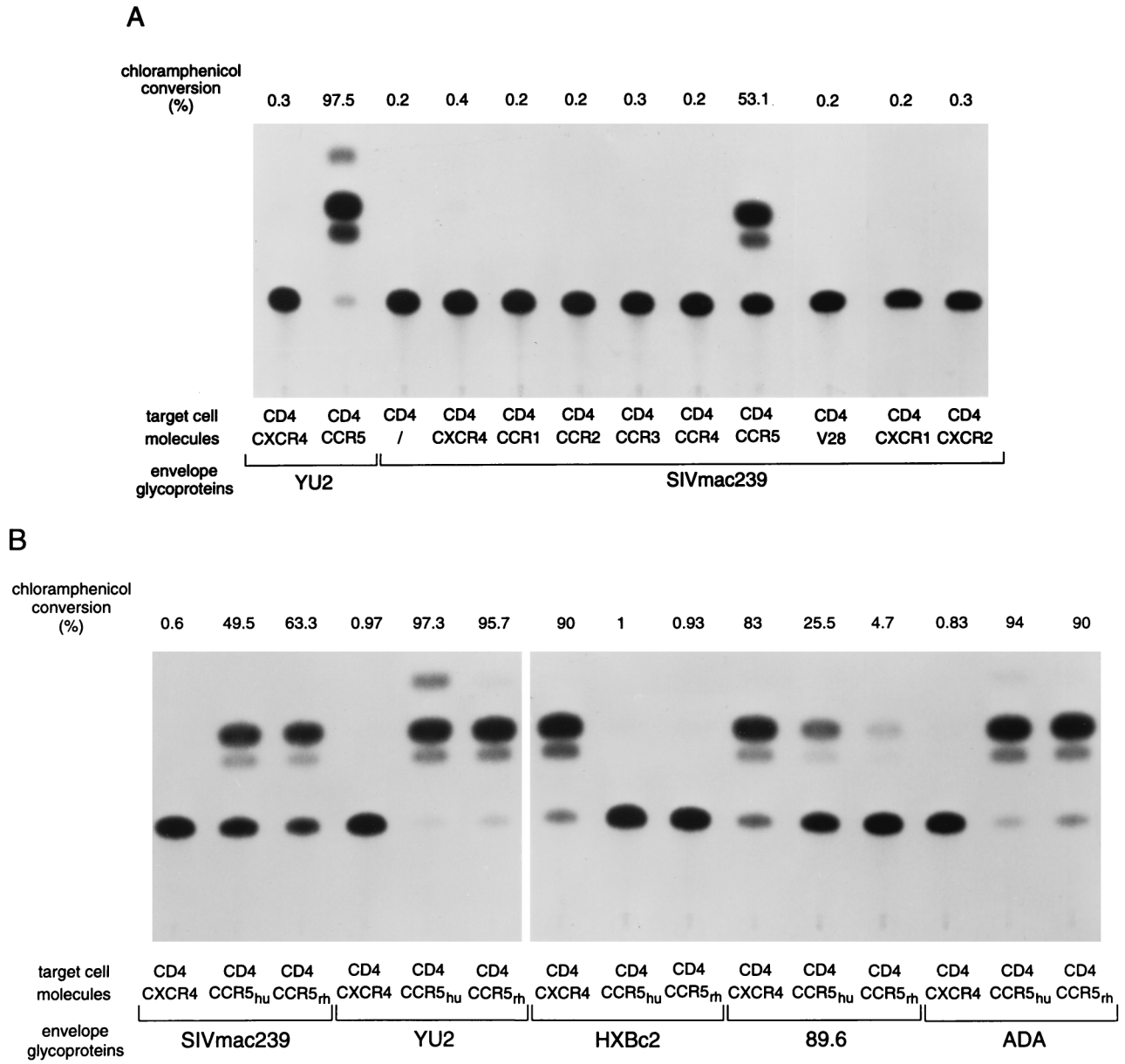


FIG. 1. CAT activity in Cf2Th cells expressing CD4 either alone or together with the various chemokine receptors after incubation with HIV-1 recombinant viruses carrying the $SIV_{mac}239$ or the HIV-1 YU2, HXBc2, 89.6, or ADA envelope glycoproteins. The percentage of acetylated chloramphenicol from a representative experiment, in which a portion of the cell lysate containing 20 μg of protein was used, is shown. (A) CAT activity in cells expressing CD4 and the various human chemokine receptors. (B) CAT activity in cells expressing CD4 and CXCR4, CD4 and $CCR5_{hu}$, or CD4 and $CCR5_{rh}$.

coprotein changes were reported to mediate their effects at levels of virus replication other than virus entry (49). Nonetheless, studies of human and animal cells transfected with human CD4 indicated that target cell factors in addition to CD4 are important for SIV entry (13, 37a, 47). Some changes in the $SIV_{mac}239$ V3 region can result in target cell-specific changes in virus entry (35b). In addition, some SIV isolates can be inhibited by RANTES, MIP-1α, and MIP-1β, suggesting the possibility that SIV, like HIV-1, might utilize members of the chemokine receptor family for entry (15). Here we test this hypothesis, using the envelope glycoproteins of a molecularly cloned $SIV_{mac}239$ isolate that induces an AIDS-like disease in monkeys (35).

To determine whether the $SIV_{mac}239$ envelope glycoproteins could utilize chemokine receptors as cofactors for entry into $CD4^+$ cells, an *env* complementation assay was used (12, 28). Recombinant virions were generated by cotransfecting two plasmids, the pHXBH10ΔenvCAT plasmid and a plasmid expressing either the $SIV_{mac}239$ or HIV-1 envelope glycoproteins. The pHXBH10ΔenvCAT plasmid contains an HIV-1 provirus carrying a deletion in the envelope gene and the chloramphenicol acetyltransferase (CAT) gene instead of the *nef* gene. Recombinant virions were then used to infect target cells expressing human CD4 in conjunction with different human chemokine receptors. CAT activity was measured in the target cells 60 h after infection. The CAT activity reflects the efficiency of the early steps of retroviral infection of the target cells.

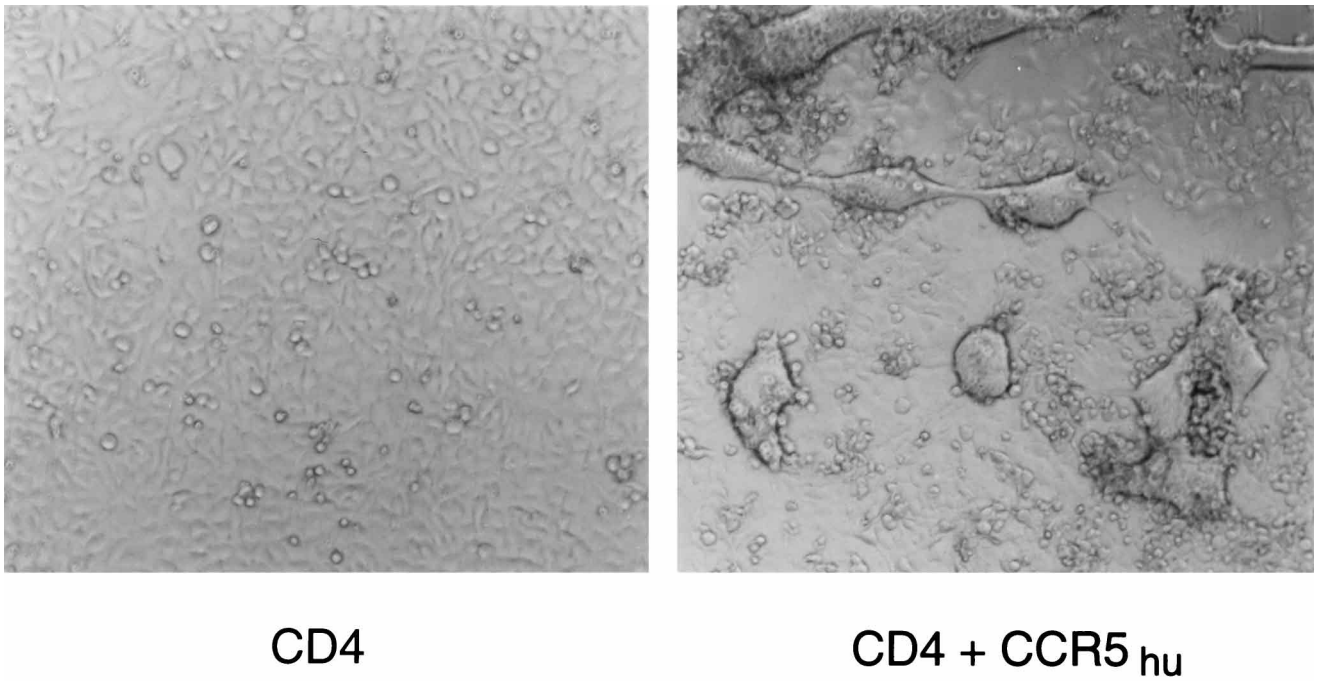


FIG. 2. Syncytium formation mediated by the $SIV_{mac}239$ envelope glycoproteins in HeLa cells expressing CD4 either alone or with $CCR5_{hu}$. HeLa cells expressing CD4 alone (left panel) or together with $CCR5_{hu}$ (right panel) were cocultivated for 20 h at 37°C with cells expressing the $SIV_{mac}239$ envelope glycoproteins. Magnification, ×200.

Recombinant virus was generated by cotransfecting HeLa cells with pHXBH10ΔenvCAT DNA and either pSIVΔgpv, which expresses the $SIV_{mac}239$ envelope glycoproteins (45a), or pSVIIIenvYU2, which expresses the envelope glycoproteins of a macrophage-tropic primary HIV-1 isolate (12, 61a), by the calcium phosphate technique. Supernatants were collected 60 h after transfection and centrifuged at 470 × *g* for 15 min to remove cells, and reverse transcriptase activity was then measured. A canine thymic cell line, Cf2Th, was chosen as the target cell line. Cf2Th cells were transiently transfected with plasmid DNA (pCD4) expressing the human CD4 molecule alone or together with plasmids expressing human CCR1, CCR2, CCR3, CCR4, CCR5, CXCR4, V28, CXCR1, or CXCR2 (12). Expression of these molecules at the cell surface has been previously documented (12). The recently cloned rhesus monkey CCR5 cDNA (27a) was also expressed along with human CD4 in the Cf2Th target cells. Equal numbers of reverse transcriptase units, ranging between 25,000 and 40,000 cpm, of recombinant viruses with the $SIV_{mac}239$ or YU2 envelope glycoproteins were used to infect Cf2Th cells expressing CD4 and the various chemokine receptors. Sixty hours after infection, the cells were lysed and CAT activity was measured in a portion of the cell lysate, after normalization for protein content with the Micro BCA protein assay (Pierce).

Recombinant virions containing the $SIV_{mac}239$ envelope glycoproteins were able to infect Cf2Th target cells expressing CD4 only when human CCR5 was also present (Fig. 1A). None of the other human chemokine receptors tested served as an efficient cofactor for entry of this recombinant virus. Both the simian ($CCR5_{rh}$) and the human ($CCR5_{hu}$) CCR5 molecules supported efficient infection by the recombinant viruses containing $SIV_{mac}239$ envelope glycoproteins and various primary HIV-1 envelope glycoproteins (Fig. 1B). Recombinant viruses carrying the HXBc2 and 89.6 envelope glycoproteins were able to infect Cf2Th cells expressing CD4 together with the CXCR4 molecule, as previously reported (12, 20, 24).

Many immortalized human cells, such as HeLa, do not express CCR5 and are therefore resistant to infection by macrophage-tropic primary HIV-1 isolates (1, 10, 12, 17, 20, 21). Since the expression of human CD4 alone is not sufficient to support fusion of cells expressing the $SIV_{mac}239$ envelope glycoproteins with HeLa cells, we tested whether the concomitant expression of CD4 and CCR5 would render the HeLa cells permissive for fusion. To assess the fusion ability of the $SIV_{mac}239$ envelope glycoproteins, a syncytium formation assay was used. Envelope glycoprotein-expressing cells were cocultivated with target cells expressing CD4 and the chemokine receptors. HeLa cells were transfected with pCD4 and plasmids encoding the human chemokine receptors CXCR4, CCR1, CCR2, CCR3, CCR4, and CCR5. In parallel, HeLa cells were transfected with pCD4 and the simian $CCR5_{rh}$-expressing plasmid. Forty-eight hours after transfection, cells were detached with 5 mM EDTA in phosphate-buffered saline. Cells were then washed with phosphate-buffered saline, resuspended in medium, and counted. A 10- and 20-fold excess of these cells was then cocultivated with $1 \times 10^4$ to $5 \times 10^4$ HeLa cells that had been transfected 48 h earlier with the pSIVΔgpv plasmid expressing the $SIV_{mac}239$ envelope glycoproteins. The HeLa cells expressing the $SIV_{mac}239$ envelope glycoproteins were able to form syncytia only when CD4 and either $CCR5_{hu}$ or $CCR5_{rh}$ were coexpressed on the HeLa target cells (Fig. 2 and 3). None of the other chemokine receptors, when coexpressed with CD4, supported syncytium formation mediated by the $SIV_{mac}239$ envelope glycoproteins (Fig. 3). No significant difference was observed between the numbers of syncytia present when the $CCR5_{hu}$ or $CCR5_{rh}$ molecules were coexpressed with CD4 in the target cells.

The identification of CCR5 as an entry cofactor for

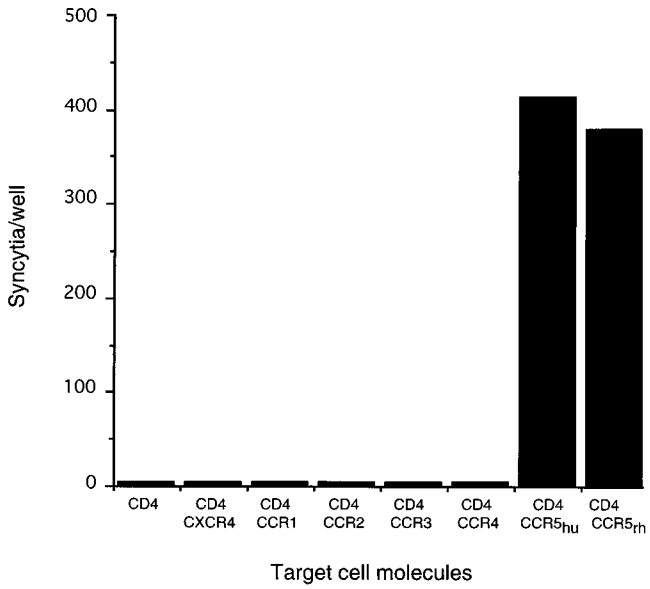


FIG. 3. Syncytium formation in HeLa cells expressing CD4 either alone or together with various chemokine receptors after cocultivation with cells expressing the $SIV_{mac}239$ envelope glycoproteins. The numbers on the ordinate indicate the number of syncytia counted in a well of a 24-well plate 20 h after cocultivation. The values shown are the results of a representative experiment.

$SIV_{mac}239$ is consistent with the observation that some SIV strains are inhibited by RANTES, MIP-1α, and MIP-1β (15), the ligands for CCR5 (54, 57). This observation also indicates that other SIV isolates are likely to use CCR5 for infection. Although the evolution of the $SIV_{mac}239$ virus to a macrophage-tropic variant involved changes in *env*, these changes did not apparently effect an increased efficiency of entry into these cells (48, 49). Since CCR5 is known to be expressed on human monocytes/macrophages (1, 21) and since this chemokine receptor is utilized by primary HIV-1 isolates for infection of these cells (1, 12, 17, 20, 21), it is likely that CCR5 can be utilized for entry by both $SIV_{mac}239$ and the macrophage-tropic variants. It appears that the observed differences in *env* in these viruses specify other cell type-dependent properties. Further work will be required to gain an understanding of these properties and to determine if the expression of entry cofactors accounts for some of the reported differences in the abilities of cell lines to support HIV-1, HIV-2, and SIV entry and fusion (13, 37a, 47).

The use of the same chemokine receptor, CCR5, as an entry cofactor by viruses as diverse as $SIV_{mac}$ and the macrophage-tropic primary HIV-1 isolates is noteworthy. If, during virus entry, interactions between CCR5 and the viral envelope glycoproteins occur, they may involve relatively well-conserved structures. This must be reconciled with genetic data suggesting that the rather variable gp120 V3 sequence specifies chemokine receptor use by HIV-1 (12). Conserved structures in the V3 loop that are not apparent from direct examination of the primary amino acid sequence might interact with CCR5. In this respect, it is interesting that the V3 regions of both primary HIV-1 isolates and $SIV_{mac}$ have been suggested to be less accessible to antibodies and proteases than has the same region of laboratory-adapted HIV-1 (6, 6a, 31, 61a). Alternatively, the ability of conserved envelope glycoprotein structures outside of the V3 loop to bind CCR5 might be influenced by the conformation of V3. It is also theoretically possible that completely different HIV-1 and $SIV_{mac}$ envelope glycoprotein regions mediate the interaction with CCR5. Future work should distinguish among these possibilities and identify any envelope glycoprotein elements interacting with the chemokine receptors.

Human and simian CCR5 molecules are very closely related, with only four amino acid differences in the extracellular sequences of these proteins (27a). Both can serve as entry cofactors for HIV-1 and SIV, consistent with previous studies indicating that species-specific differences in HIV-1 and SIV replication are not determined at the level of virus entry (29, 33, 41, 49, 58).

Monkeys infected with $SIV_{mac}239$ exhibit two patterns of AIDS pathogenesis: rapid disease induction (in less than 6 months) or a slower course of disease induction (occurring over a 1- to 3-year period following infection) (18, 35, 35a, 40). In humans, mutant alleles of the CCR5 gene have been shown to contribute to resistance to HIV-1 infection or slower disease progression (16a, 43, 52, 57a). Future studies might address whether variations in CCR5 structure or expression represent host variables that influence the outcome of primate lentivirus infections.

**Nucleotide sequence accession number.** The GenBank accession number for the rhesus monkey CCRS cDNA is U77672.

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